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COMPARATIVE STUDIES ON RESPIRATION XXI.

ACID FORMATION AND DECREASED PRODUCTION OF CO_2 DUE TO ETHYL ALCOHOL

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The purpose of the present investigation is to determine the effect of ethyl alcohol on the production of carbon dioxide by seedlings of the radish (Early French Breakfast).

The seeds were allowed to germinate on moist filter paper until the caulicles were about 5 mm. long: they were then used for the experiments. The apparatus used for the determination of carbon dioxide production is described in a previous paper.[1] It is important first to test the apparatus for leakage and to allow the air to pass through the tube containing the lumps of sodium hydroxide until the whole apparatus is practically free from carbon dioxide. The tube is then disconnected and the organisms are placed in it. It is important that a check experiment, without the seedlings, should be made to ascertain whether the entrance of the laboratory air on disconnecting the tube introduces any error. After the seedlings are placed in the tube and the whole apparatus is again connected, the air is again pumped through a tube containing sodium hydroxide for three minutes to absorb the carbon dioxide which is introduced when the tube is disconnected.

The period during which the liquid containing the indicator changes from pH 8 to pH 7.6 is recorded by a stop watch, and the reciprocal of this time is taken as the rate of production of carbon dioxide by the seedlings.

The color of the indicator is affected by alcohol, which makes it necessary to correct the readings by calibrating the indicator solutions which have taken up alcohol vapor by adding to them measured amounts of carbon dioxide. This must be done for each concentration of alcohol.

In order to ascertain the normal rate of respiration, seedlings are placed in tap water and readings are taken every ten minutes for three hours. Curve *A*, figure 1, shows that a series of readings of this sort agree, and it is evident that the experimental errors are not large. An average of the

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[1] Irwin, M. Jour. Gen. Physiol. 3 : 203–206. 1920.

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whole series is taken, and this is considered to be 100 percent; with this average as the standard, the relative rate of production of carbon dioxide under the influence of alcohol is calculated.

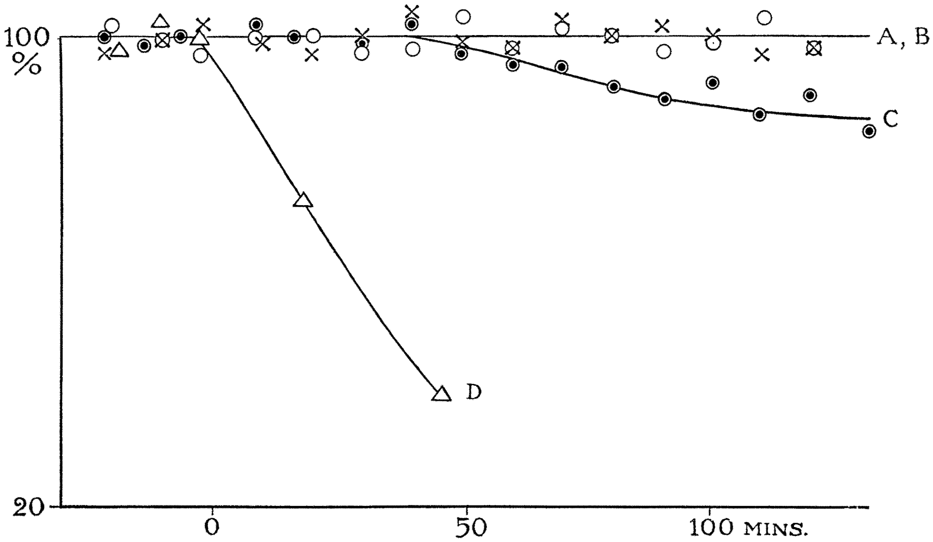

FIG. 1. Curves showing the effect of ethyl alcohol on the production of carbon dioxide by radish seedlings. To the left of the point O on the abscissa the curve shows the rate of respiration in tap water; to the right of that point, the rate under the influence of alcohol, except in curve *A* which represents the rate in tap water (without exposure to alcohol). Curve *B* shows the rate in 1*M* alcohol, curve *C* in 2*M* alcohol and curve *D* in 4*M* alcohol. Each point represents an average of 12 experiments. Probable error of the mean is less than 3 percent of the mean.

Before exposing the seedlings to a solution of alcohol, control readings are made with the seedlings in 3 cc. of tap water. The seedlings are then taken out of the apparatus and kept in a large dish of water. A bottle with a capacity of 250 cc., filled with a solution of alcohol, is fitted to the portion of the apparatus to which the tube which is to contain organisms is usually fitted. The liquid containing the indicator is now replaced by the same amount of liquid of the same concentration of alcohol as that contained in the larger bottle and having the same concentration of indicator. The whole apparatus is thus saturated with the alcohol vapor in equilibrium with the solution which is to be used for the experiment. Then 3 cc. of alcohol of the same concentration is placed in the tube and the air is again passed through the U-tube containing sodium hydroxide until the whole apparatus is again free of carbon dioxide. The organisms are now placed in the tube and the air is passed through the sodium hydroxide for three minutes, after which the reading is made in the same manner as in the control experiment. The relative rate of respiration of the seedlings in alcohol is calculated on the basis of the average of the control experiment, which was made immediately before the experiment with the alcohol.

With 1*M* alcohol the production of carbon dioxide by seedlings remains unchanged for the first hour, as shown by figure 1, curve *B*. With 2*M* alcohol there is a slight decrease during the first hour (fig. 1, curve *C*). With 4*M* solutions, a great decrease takes place in about twenty minutes (fig. 1, curve *D*). This change is irreversible.

The fact that death is often accompanied by an increase in the production of CO_2 might lead to the suspicion that such an increase also occurs in the case of alcohol.

With the apparatus used for the present investigation, the first reading cannot be taken within ten or fifteen minutes after the immersion of the seedlings in the test solution. For this reason it might be supposed that an increase followed by a decrease may have taken place during the first fifteen minutes of the experiment. If an increase really occurred then, it seems probable (on the basis of other experiments) that it would persist for a time after removal from the alcohol. In order to test this, the seedlings were put in 4*M* alcohol, removed and washed for half a minute in running water, and then put into the apparatus with 3 cc. of tap water, after which the readings were made. Exposure of radish seedlings to the alcohol for three minutes brought about no change in the rate of respiration, while there was a slight decrease after an exposure of five minutes, and a greater decrease after exposures of seven and ten minutes, as shown in figure 2, curves *B*,

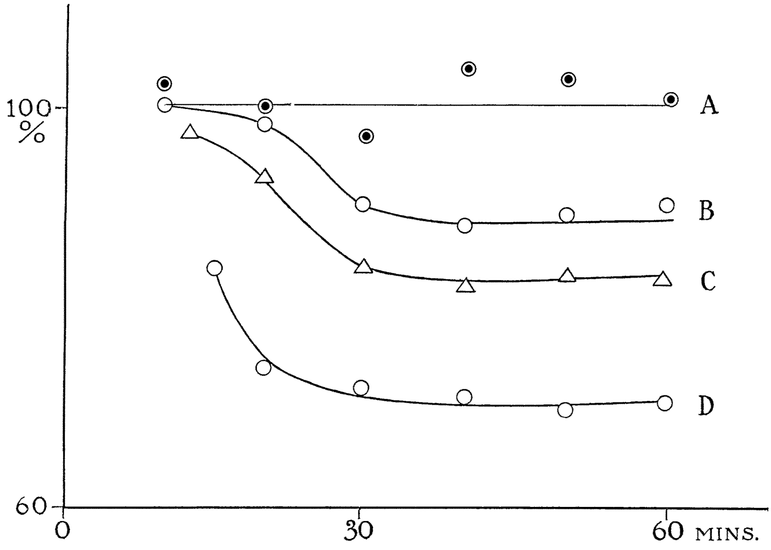

FIG. 2. Curves showing the effects of 4*M* ethyl alcohol on the production of carbon dioxide by radish seedlings. Curve *A* shows the rate of respiration in tap water (without exposure to alcohol). Curve *B* shows the rate after the seedlings have been exposed to the alcohol for 5 minutes and then replaced in tap water; curve *C*, exposed for 7 minutes; curve *D*, for 10 minutes. Each point represents the average of 12 experiments. Probable error of the mean is less than 4 percent of the mean.

C, and *D*. These experiments seem to indicate that alcohol does not produce an initial increase in the rate of production of carbon dioxide.

After experimentation the seeds were allowed to grow on wet filter paper in a moist chamber in sunlight. If they had been exposed for ten minutes to 4*M* alcohol, there was no subsequent growth. After an exposure of five minutes there was slight growth of the root tip and considerable (though retarded) growth of the stem. After an exposure of three minutes the retardation of growth was very slight.

It is difficult to make any comparisons between growth and the production of carbon dioxide for the reason that the susceptibility of the different parts of the seedlings varies. The root tip is most sensitive, so that a short exposure may influence its growth considerably, but it represents only a small portion of the seedling, so that its injury may not make much difference in the production of carbon dioxide if the remaining, less susceptible portion is left unaffected. A great decrease in the production of carbon dioxide takes place only when the entire seedling is affected to such an extent that there is no subsequent growth.

When the seedlings are exposed to 4*M* solutions of alcohol for about five minutes, formation of acids other than carbonic takes place. This may be detected by the following method. Twenty seedlings were put into a glass-stoppered Pyrex tube with 3 cc. of tap water (containing the same amount of indicator as in the standard tube) for twenty minutes; 2 cc. of this liquid were then put into the indicator tube, and air free from carbon dioxide was passed into the tube just as in the previous experiments. The time required to bring the liquid to pH 7.6 by thus driving carbon dioxide out of the liquid was about three minutes. The same seedlings were now placed in a glass-stoppered tube containing a 4*M* solution of alcohol, washed for one half minute in running water and placed in 3 cc. of tap water for twenty minutes. 2 cc. of this liquid were then placed in the indicator tube, and the air free from carbon dioxide was passed through it to drive out the carbon dioxide. After three minutes' exposure of the seedlings to 4*M* alcohol, the time recorded was about the same as in the control. This indicates that there is no acid other than carbonic in the liquid.[2] But when the seedlings had been exposed for five minutes to the same concentration of alcohol the change was very much slower; in many cases the change did not take place within ten minutes. After ten minutes there was no change within twenty minutes. This proves that acids, other than carbonic, must be present in the liquid. The slowness of the change back to pH 7.6 cannot be due to excess of carbon dioxide, since the previous experiments show that less carbon dioxide is produced in 4*M* alcohol.

The question arises whether the presence of such acids is due to the extraction by alcohol of acids already present before the experiment was begun, or whether it is due to the actual formation of such acids as a direct effect of the alcohol on the cells. It was found that when seedlings were killed in boiling water the presence of the acids could be demonstrated. Since enzymes are generally destroyed at this temperature, this experiment might seem to show that the acids were already present in the uninjured cells. However, in the case of oxidizing enzymes it has been shown that it is very difficult to destroy the enzymes by this method rapidly enough to prevent the oxidation completely. This is readily demonstrated in the Indian pipe (*Monotropa uniflora*), in which case it is difficult to destroy the enzymes rapidly enough by immersing the plant in boiling water to prevent the appearance of the black pigment.

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[2] When other volatile acids are absorbed by water, it is difficult to drive the acid out of the water by a current of air and bring the water back to neutrality.

If the acids are normally present, it would be expected that they could be detected by killing the seedlings by liquid air. But when seedlings were exposed to liquid air and then crushed and extracted with cold water, there was no evidence of the presence of these acids.

Seedlings were allowed to dry for one month on filter paper at room temperature and were then crushed and extracted with cold water, and the acids were found. The production of carbon dioxide by the dried seedlings, after they were replaced in water, was found to be about one half the normal. Growth occurred when the seedlings were again soaked in water, but it was somewhat retarded.

It is difficult to draw definite conclusions from these experiments, but it is possible that ethyl alcohol accelerates the decomposition of certain substances, forming an excess of intermediate products in the form of organic acids, until the concentration of the hydrogen ion (or of some other substance) increases to such an extent that any further decomposition into carbon dioxide and water is partly inhibited. The nature of the organic acids has not been determined, but further investigations may throw light on this point.

The question may be raised whether the production of acids other than carbonic is due to the oxidation of the alcohol. Oxidation[3] of alcohol by higher plants has been reported. But in the experiments described above oxidation of ethyl alcohol does not seem to be the chief cause of the formation of organic acids, because, as it was already shown, organic acids were formed when the seedlings were put in boiling water without having been treated with alcohol.

It may be of interest to note the extreme complexity of the problems of respiration as indicated by the difference in the behavior of the radish seedlings in ethyl alcohol, as contrasted with the behavior of Salvia in ether.[4] In the case of radish seedlings production of carbon dioxide is decreased and the acidity of the tissue apparently increases, while in the case of Salvia production of carbon dioxide is increased and the alkalinity increases.

Loeb and Wasteneys[5] showed that ethyl alcohol decreases the consumption of oxygen by *Strongylocentrotus purpuratus*, but that this is not sufficient to account for the narcotic effect on the basis of decrease of oxygen consumption (though a decrease of about sixteen percent of the normal is brought about). They point out that this is in disagreement with Verworn's theory. The experiments of the writers are in harmony with this conclusion.

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[3] Zaleski, W. Biochem. Zeitschr. 69 : 283–289. 1915.

[4] Irwin, M. Jour. Gen. Physiol. 1 : 399–403. 1919.

[5] Loeb, J., and Wasteneys, H. Jour. Biol. Chem. 14 : 517–523. 1913.

SUMMARY

Ethyl alcohol decreases the production of carbon dioxide by radish seedlings and at the same time brings about the formation of organic acids.

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